GENERAL LIBRARY
JUN 25 1920
UNIV. OF MICH.

VOL. 47 JUNE 1920 NO. 6

BULLETIN

OF THE

TORREY BOTANICAL CLUB

Editor

ALEXANDER WILLIAM EVANS

Associate Editors

JEAN BROADHURST
JAMES ARTHUR HARRIS
MARSHALL AVERY HOWE
MICHAEL LEVINE
GEORGE ELWOOD NICHOLS
ARLOW BURDETTE STOUT
NORMAN TAYLOR

CONTENTS

Microsporogenesis in Datura Stramonium. (Plates 8 and 9). CLAUDE E. O'NEAL 231

The refraction of light in plant tissues . . . . . FOREST B. H. BROWN 243

The physical factors in the growth of the tomato . . . . D. T. MacDOUGAL 261

INDEX TO AMERICAN BOTANICAL LITERATURE 271

PUBLISHED FOR THE CLUB
THE NEW ERA PRINTING COMPANY
LANCASTER, PA.

That Camera You Have Long Wished For

Get it from Bass at the price you can pay. Fair allowance for old cameras.

We make a specialty of scientific laboratory equipment, especially for microbe and plant photography, for lantern slide or motion picture projection.

Expert advice willingly given free of all obligations.

Advise us of your needs at once.

Bass Camera Company

109 N. Dearborn St. Dept. B CHICAGO, ILLINOIS

THE BRYOLOGIST

PUBLISHED BY THE

SULLIVANT MOSS SOCIETY

The only magazine in English devoted wholly to Mosses, Hepatics, and Lichens. Bimonthly; amply illustrated; for beginners as well as for advanced students. Yearly subscription, $1.25. Address

EDWARD B. CHAMBERLAIN,

18 West 89th Street, NEW YORK CITY.

Illustrations by Photomicrography

Photomicrographs and lantern slides made of vegetable and animal tissues and other substances to illustrate original scientific papers, textbooks, lectures, and popular articles, advertising, etc. Materials and manufacturing processes compared by photomicrographic methods.

Color screens used to preserve correct color values and also to increase contrast and bring out any particular structure.

Specimens prepared for photographing.

All magnifications. Size of field unlimited. Zeiss apochromat oculars and Zeiss compensating eyepieces used.

A. TENNYSON BEALS 2929 Broadway, NEW YORK
Phone Gramercy 2271

Please mention the "Bulletin" when answering advertisements.

Vol. 47 No. 6

BULLETIN

OF THE

TORREY BOTANICAL CLUB

JUNE, 1920

Microsporogenesis in Datura Stramonium

CLAUDE E. O'NEAL

(WITH PLATES 8 AND 9)

It has long been known that the representatives of the genus *Datura* furnish interesting material easily available for plant experimentation. In addition, it has been pointed out recently (Blakeslee & Avery **2**, **3**) that this genus also affords an excellent field for the study of Mendelian inheritance and mutative variation. The general morphology of the members of the genus is a point of common knowledge, but so far as the writer is aware, no careful cytological studies have been made upon the reduction divisions of any of its members. Guignard (**7**) in his now classical work treats of double fecundation in *D. laevis*. Bönicke (**4**) has examined the prophases and counted the chromosomes in *D. Stramonium*. Aside from these two investigations, the work that has been done upon *Datura* has been of a pharmaceutical or of an experimental character.

The abundance of specimens of *D. Stramonium* near at hand during the summer of 1915 led the author to prepare a large number of anthers for a study of microsporogenesis in this plant. The material was killed, fixed, and embedded in paraffin, in which condition it was kept until the summer of 1919, when an increased interest in the plant as a teaching type induced the belief that a completion of the work and a publication of the results would be of interest. Accordingly, during the months of June and July,

[The BULLETIN for May (47: 199–230. *pl. 6, 7.*) was issued June 3, 1920.]

additional material was secured and the study of the problem renewed.

Material.—The 1915 collection was made from the purple-colored, spiny-fruited plants found growing in an abandoned barnyard near Delaware, Ohio; that collected in 1919 came from plants growing on the Indiana University campus and in a neighboring garden. The plants from both of the latter places were from seed furnished by A. F. Blakeslee. Those in the garden plot were known to be homozygous for the dominant characters, purple color and spiny fruit. The plants on the campus had come about through a series of rather indiscriminate crosses between the purple, spiny-fruited type and the green, smooth-fruited plants described by Blakeslee (2). In making collections from this plot care was taken to select flowers from plants showing the two dominant characters, but since the ancestry was not definitely known, some anthers used may have been taken from heterozygous plants. A careful consideration of the stained cells from the three sources failed to show any appreciable distinction and no attempt was made to keep the preparations separate, but most of the work was done with the Blakeslee strain.

Method.—Parts of the corollas bearing stamens with anthers in various stages of development were killed in the chromo-osmo-acetic and chromo-acetic solutions, washed, dehydrated, embedded in paraffin, and cut into sections 3–5 μ thick. The killing fluid containing osmium gave uniformly better fixation and, in the latter part of the work, its use was adhered to strictly. Haidenhain's haematoxylin and the modified triple stain were used. The orange G of the latter combination was used in clove oil as recommended by Chamberlain and others.

Adaptability of material.—The pollen mother cells of *Datura* offer fair conditions for cytological investigation. Since the anthers in a bud mature their pollen simultaneously, it is necessary to examine a large number to secure the various stages. Some difficulty is attached to the removal of the young cells for the preliminary examination. This probably explains why they have not been examined cytologically before. The sporogenous cells are rather small and have very dense cytoplasm, but the chromatin material is fairly abundant for a dicotyledonous plant. Com-

paratively few chromosomes are formed and the cells stain readily with the usual laboratory dyes.

Anthers.—When the sporogenous cells are developing, the anthers range from two to four millimeters in length; those six millimeters long almost invariably contain mature pollen. Each anther has four loculi, the walls of which are composed of four layers of flat cells. As the anthers mature the cell walls of the two middle layers of these cells become thickened in the fashion usual for mechanical tissue and finally aid in the liberation of the pollen grains. The fourth layer becomes modified to form the tapetum which immediately surrounds the sporogenous cells.

Tapetal cells.—The cells of the tapetum are about the same size as the pollen mother cells when the latter are in the resting condition. At this time they are uninucleate, but by the time the mother cells have reached the spirem stage, they usually contain two or more nuclei, which have arisen from the original one by fragmentation. The tapetal cells increase in size as the spore-producing tissue develops, sometimes attaining a diameter two or three times that of the sporogenous cells. During this development the cytoplasm of most of the cells becomes decidedly vacuolated and, as it continues, the majority of the cell walls break down, and the cell contents are allowed to escape into the pollen cavity. Some of the cells apparently become reduced in size without any evident breaking of the walls, and still others seemingly persist unchanged until the pollen is matured.

The periplasm was carefully searched for "wandering cells" such as were found by Duggar (**6**) in *Symplocarpus* and more recently in *Galium* by Juel (**8**), but no positive evidence of such was found. Occasional cells were observed which had apparently broken loose from the anther wall. These were always angular in outline and were attributed to faulty manipulation rather than to any evidence of vital phenomena. Tapetal nuclei are not abundant in the periplasm; sometimes they may be seen as elongate, irregularly shaped structures, similar to those described by Pickett (**13**) for *Arisaema*, but more frequently as mere darkly staining fragments. No amoeboid forms were found.

Sporogenous cells.—The pollen mother cells arise directly from the primary sporogenous cells without division (Coulter & Cham-

berlain, 5); this accounts for their comparatively small number. In the resting stage they are about 25 μ in diameter and have large prominent nuclei. The cytoplasm is very dense and in the stained preparations seems to be composed of minute particles closely placed. As development proceeds these particles become more or less clumped and arranged in strands as shown in the figures. No evidence of mitochondria or other extra-nuclear bodies was found, but it is not doubted that such might be revealed if proper fixing and staining methods were used.

Nucleus.—In the so-called resting stage the nucleus has a diameter about two thirds that of the containing cell. It usually has a single darkly staining nucleolus, but occasionally two are found. The linin reticulum is composed of very delicate fibers upon which the chromatin material is arranged in the form of minute bodies (FIG. 1). In the preparations the threads are sometimes broken or else do not take the stain properly, but there seems to be no regular arrangement of either the threads or the chromatin bodies upon them. The material collected was too far advanced to show the telophase of the preceding division, but to all appearances the chromosomes of this phase lose their individuality in the maze of fibers and very small and numerous granules seen in the resting stage. It would seem impossible to trace a definite set of these fibers and chromatin masses to a particular chromosome, as Nothnagel (11) was able to do in the case of *Allium*.

FROM RESTING STAGE TO SPIREM

The synaptic condition is reached while the anthers are still quite young (FIG. 4). The initiation of this stage is marked by a thickening and consequent contraction of the linin threads (FIG. 2), which when continued results in their withdrawal from the periphery of the nucleus as shown in FIG. 3. Before the thickening has proceeded far the threads stain readily and their ramifications may be made out easily. The stage represented by FIG. 2 was studied carefully for evidence of the two spirem threads reported by Bönicke (4) for this plant. It will be noticed that the threads apparently anastomose freely and occasionally approximate as shown in the middle of the figure. Whether or not the two ap-

proximating threads occasionally seen should be considered spirem threads in the sense that Bönicke uses the term the writer is unable to determine. This approximation cannot be observed in every cell in this stage of development; it is more in evidence in the figure than is ordinarily seen. In other parts of the same nucleus the threads seem to radiate from chromatin masses, and in still other places the threads seem to be formed by the union of three or more smaller ones. To the mind of the writer the effect is so confusing, that he feels that anyone with a bias in his thinking, no matter what it might be, could find an apparent basis for it here. FIG. 3 shows a stage a little later than that shown in FIG. 2. The linin-chromatin mass has withdrawn from the nuclear membrane and is enveloping the nucleolus. In case two nucleoli are present one of them may be left out of the synaptic ball. At this time the threads and chromatin masses have increased greatly in thickness and stain sharply. On account of the contracted condition the arrangement can not be made out so readily as in the stage just preceding.

Synapsis.—This term is used in botanical literature for the condition shown in FIG. 4. The nucleolus is closely enveloped in the chromatin mass, but its outline as well as that of the threads may be distinguished. The details of the threads, however, can not be made out even in the most carefully stained preparations. This stage occurs so regularly and the sequence is so evident that it is hard to believe that it is an artifact, as Schaffner (14) asserts for *Agave*. In it the formation of the spirem thread is consummated.

Hollow spirem.—FIG. 5 shows the spirem thread as it emerges from the synaptic ball. At this time it is rather thin and when stained with suitable density it appears granular in nature. If the stain is dense, it appears as a fairly smooth thread without any internal differentiation whatever. At this stage no evidence of the double nature of the thread could be made out. Later the thread becomes more loosely arranged in the nuclear cavity as shown in FIG. 6. So far as it has been possible to determine, it is endless.

FORMATION OF BIVALENTS; METAPHASE; ANAPHASE; TELOPHASE

The stage represented in FIG. 6 is followed by a contraction and consequent thickening of the strand. The slightly thickened

strand has a tendency to become arranged in loose loops, which are cut off to form bivalents. In this behavior *Datura* is strikingly similar to *Lilium* as described by Mottier (**9**). FIG. 7 represents an early segmentation stage. It may be observed from this figure that all of the bivalents are not formed by the cutting off of loops from the spirem thread but that some of them must arise from the straight loopless part of it. Shortly before the segmentation of the chromosomes is brought about, the double nature of the thread becomes evident for the first time (FIG. 11). When segmentation is complete the twelve bivalent chromosomes may be made out. These bivalents may assume a number of different shapes as shown in FIGS. 9 and 10. Some of them appear as closed rings, others as U's, and still others have the chromosomes twisted about each other. The method of forming these various types is easily seen. The loops cut off from the spirem (FIG. 7) form the rings by the union or overlapping of their ends. If the ends are not united or overlapped the U-shaped form results, while the twisted form of bivalent results from the contraction of a loop with a twist in it. Payne (**12**) has shown that in the European earwig ring-shaped chromosomes may arise in three different ways in the same individual or even in the same cell. So far as it has been possible to determine, the method given above is the only one followed in *Datura*. In *Agave* (**14**) Schaffner was able to find a constant number of the various types of chromosomes present. A careful consideration of the cells of *Datura* fails to show any regularity in this respect.

Preceding the metaphase the bivalents appear in the multipolar arrangement so often described. This stage is of very short duration and difficulty is experienced in securing it. In the second division it is much more evident (FIGS. 16, 17). In the metaphase the bivalents become arranged in the usual fashion in the middle of the cell. In a polar view they are seen to have a regular distribution (FIG. 13). In this stage the chromosomes appear more or less heart shaped when viewed from the side (FIG. 12) and closely resemble Mottier's figure for *Helleborus* (**10**). There is no separation of the two halves of the chromosomes on the way to the poles, although the longitudinal division has doubtless taken place (FIG. 11). FIG. 14 shows a telophase stage with the chromosomes

still undivided. The volume of the chromatin material is considerably reduced between the segmentation of the chromosomes and their separation in the anaphase (FIGS. 6, 7, 8, 9, 11). As usual in the dicotyledonous plants a cell wall is not laid down till after the second division (FIG. 18).

SECOND DIVISION; MICROSPORES; POLLEN GRAINS

The material from which this study was made was taken from plants that were growing rapidly, and in it the chromosomes do not become diffused nor in any way lose their individuality between the first and second divisions. It sometimes happens that they become arranged to form a sort of a broken spirem, but each individual may be made out in it easily. This does not seem to occur with any regularity and the relation established is an approximation rather than a fusion of material. Whether or not the chromosomes behave any differently when growth is less rapid has not been determined. FIG. 15 shows a stage slightly further advanced than that represented by FIG. 14. Here the double nature of the chromosomes due to longitudinal splitting or separation is very evident. The next step in the process is a breaking down of the nuclear membrane and the formation of a typical multipolar spindle. When this multipolar condition first appears the chromosomes are seen to be more or less clumped in the midst of the weft of fibers. At this time (FIG. 16) the double nature of the chromosomes is somewhat obscured, but soon they become separated somewhat and their true nature may be made out easily (FIG. 17). In the bipolar spindle which soon follows, the chromosomes are arranged in a very exact fashion, giving the cell an almost diagrammatic appearance. In the following anaphase each chromosome is parted longitudinally and the halves started towards opposite poles. Following the telophase of this division (FIG. 18) a nuclear membrane is formed and the substance of the chromosomes again becomes arranged in the form of a reticulum similar to that seen in the resting condition of the mother cell. The material at hand does not show the details of this process. The walls separating the four cells appear about the time that the nuclear reticulum is established. The cells of the tetrads thus formed cling together for some time before separating as microspores. Shortly after

their separation the nucleus of each divides to form the generative and tube nuclei. Meanwhile the cell has increased in size greatly and a large central vacuole has been formed. A plasma membrane is formed about a portion of the cytoplasm containing the generative nucleus, thus forming the generative cell and completing the development of the pollen grain.

DISCUSSION

It is felt that anyone giving a cytological paper on a field so well-worn as that of the reduction divisions should have a distinct purpose in so doing. It is evident that such work can not reveal much that is strikingly new. A consideration of the literature of plant cytology shows that while many plants have been investigated few of the results have been correlated with the results of experimental work. Many of the plants studied cytologically have been monocotyledonous and not readily amenable to Mendelian investigation. Blakeslee (2) has shown that *Datura* has at least two pairs of characters that show Mendelian behavior with almost mathematical exactness. Such a result must be due to a precise handling of the hereditary substance and to the selection of unmistakeable characters by the plant breeder. The present work shows that there is a very definite apportionment of the chromatin material in the reduction divisions of *Datura*. After the chromosomes are cut off from the the spirem thread they may be traced as distinct individuals till the telophase of the second division. It is evident that, if the factors representing the parental characters are segregated in the two halves of the spirem thread, they would become equally distributed in the formation of the four microspores.

It has long been known that in the insects certain definite characters like sex may be traced to a specific chromosome or part of a chromosome in the reproductive cells. Recently Allen (1) has reported that a similar basis for sex may be made out in *Sphaerocarpos*, one of the liverworts. To one who has studied the cells of *Datura* it is clear that there is no visible evidence of such a basis for the Mendelian characters with which Blakeslee and Avery worked. The determinants of these characters must be chromosomes or parts of chromosomes that are for the present

indistinguishable. In this study no irregularity of chromatin behavior has been found that could explain the origin of mutants, but this could scarcely be expected since their occurrence is comparatively rare. It is conceivable that such might result from the distribution of the chromatin substance in megasporogenesis. If such is the case, it must for the present remain a closed book on account of technical difficulties and the laboriousness of the investigation.

The most striking thing about the chromosomes of *Datura* is their uniformity of size. This is especially evident in the second division. In the first division this uniformity is somewhat obscured by the shapes assumed by the bivalents until they appear in the metaphase.

Interpretation of the prophase.—The behavior of the chromatin material in the formation of the spirem thread has been a much mooted question and it seems that the present study of *Datura* can not shed much light upon it. In the resting condition the nucleus presents such a maze of threads and granules that almost any interpretation might be given. Bönicke (4) claims that the spirem thread is formed by the union of two smaller threads. The writer came to the present problem with a similar theory. As shown elsewhere the confirmatory evidence is not wholly convincing.

No satisfactory theory has been formulated as to how the chromosomes control growth or impart the parental characters to the offspring. It was purposed to make a comparative study of the chromosomes of plants showing the dominant characters and of the chromosomes from plants displaying the recessive characters. Since the rate of growth in the two types is quite different it was conceived that there might be a physical, determinable basis for it in the chromatin substance of the cells. Through an unfortunate misunderstanding the plants showing the recessive characters were destroyed before material was collected for study. Seeds have been planted, however, and it is hoped that work on this phase of the problem may be begun soon.

SUMMARY

1. As a dicotyledonous plant *Datura Stramonium* is favorable for cytological investigation as well as for studies of Mendelian behavior.

2. Part of the bivalent chromosomes are cut from the spirem thread as loops, which if twisted, result when shortened in the twisted form shown in the figures; if the ends overlap, or unite, the ring-shaped form is developed; the U-shaped type may arise from a loop or by the subsequent bending of the straight part of the thread.

3. The report of twelve bivalents made by Bönicke is confirmed.

4. There is no loss of the individuality of the chromosomes from the time that they are cut from the spirem thread till the telophase of the second division. This is an unusually striking phenomenon in *Datura*.

5. The exact results obtained by plant breeders from this plant are attributed to the unusual regularity in the formation and behavior of the chromosomes.

6. No physical basis for the Mendelian characters considered can be found in the chromosomes; nor is any cause found for the occurrence of mutants.

In conclusion, the writer wishes to take the opportunity of expressing his very great obligation to Professor D. M. Mottier, of Indiana University, under whose direction this work was brought to completion, for encouragement and valuable aid given; and to the board of trustees of the Ohio Wesleyan University for a relief from teaching duties that has made possible the pursuance of this investigation.

INDIANA UNIVERSITY

LITERATURE CITED

1. **Allen, Charles E.** The basis of sex inheritance in *Sphaerocarpos*. Proc. Am. Phil. Soc. **58**: 289–316. *f. 1–27*. 1919.
2. **Blakeslee, A. F., & Avery, B. T.** Adzuki beans and Jimson weeds. Jour. Heredity **8**: 125–133. 1917.
3. ——. Mutations in the Jimson weed. Jour. Heredity **10**: 111–120. 1919.
4. **Bönicke, L. V.** Zur Kenntniss der Prophasen der heterotypischen Teilung einiger Pollenmutterzellen. Ber. Deuts. Bot. Ges. **29**: 59–64. 1911.
5. **Coulter, J. M., & Chamberlain, C. J.** Morphology of angiosperms. New York. 1903.

6. **Duggar, B. M.** Studies in the development of the pollen grain in *Symplocarpus foetidus* and *Peltandra undulata*. Bot. Gaz. **29**: 81–98. *pl. 1, 2.* 1900.

7. **Guignard, M. L.** La double fécondation chez les Solanées. Jour. de Bot. **16**: 1–23. 1902.

8. **Juel, H. O.** Untersuchungen über die Auflösung der Tapetenzellen in den Pollensäcken der Angiospermen. Jahrb. Wiss. Bot. **56**: 337–364. *pl. 4, 5.* 1915.

9. **Mottier, David, M.** The development of heterotypic chromosomes in pollen mother-cells. Ann. Bot. **21**: 309–347. *pl. 27, 28.* 1907.

10. ——. Beiträge zur Kenntniss der Kerntheilung in den Pollenmutterzellen einiger Dikotylen und Monokotylen. Jahrb. Wiss. Bot. **30**: 169–204. 1897.

11. **Nothnagel, Mildred.** Reduction divisions in the pollen mother cells of *Allium tricoccum*. Bot. Gaz. **61**: 543–476. *pl. 28–30.* 1916.

12. **Payne, Fernandus.** Chromosomal variation and the formation of the first spermatocyte chromosomes in the European earwig, *Forficula sp.* Jour. Morph. **25**: 559–581. *pl. 1, 2.* 1914.

13. **Pickett, F. L.** Wandering tapetal nuclei of *Arisaema*. Am. Jour. Bot. **3**: 461–469. *pl. 20 + f.1–8.* 1916.

14. **Schaffner, J. H.** Reduction division in *Agave*. Bot. Gaz. **47**: 198–214. *pl. 12–14.* 1909.

Explanation of plates 8 and 9

All figures × 2270.

FIG. 1. A pollen-mother cell in resting condition.

FIG. 2. An early presynapsis stage.

FIG. 3. A stage a little later than that shown in FIG. 2.

FIG. 4. Synapsis.

FIG. 5. Coming out of synapsis.

FIG. 6. An early hollow spirem stage.

FIG. 7. Segmentation in process showing method of cutting off the bivalents.

FIG. 8. A stage a little later than that shown in FIG. 7.

FIG. 9. Segmentation.

FIG. 10. Some of the shapes assumed by the bivalents.

FIG. 11. A spirem stage in which the double nature of the thread is shown.

FIG. 12. A bipolar spindle, passing into anaphase.

FIG. 13. A metaphase, polar view, showing the twelve bivalents.

FIG. 14. A telophase.

FIG. 15. A little later stage, in which the double nature of the chromosomes is shown.

FIG. 16. An early multipolar spindle of the second division, showing the clumping of the chromosomes.

FIG. 17. A stage a little later than that shown in FIG. 16.

FIG. 18. Telophases of the second division.

The refraction of light in plant tissues*

FOREST B. H. BROWN

(WITH FOUR TEXT FIGURES)

CONTENTS

I. INTRODUCTION 243
II. METHODS OF MEASURING REFRACTION WITH THE MICROSCOPE 244
1. REFRACTIVE LIQUIDS 245
2. PREPARATION OF TISSUES FOR STUDY 247
3. DETERMINATION OF RELATIVE REFRACTION 247
A. By oblique illumination 247
B. By direct illumination 248
C. Modifications with hygroscopic tissues 248
D. Relative accuracy of the methods 249
III. REFRACTION OF TISSUES 249
1. RELATIVE REFRACTION 250
2. DEVIATION IN REFRACTION WITH IMBIBITION 250
3. CHANGE IN REFRACTION WITH GROWTH 251
IV. DEFINITION OF ANATOMICAL DETAILS 254
1. REFRACTION OF MOUNTING MEDIA 254
2. PITS AND MIDDLE LAMELLA IN COLLENCHYMA 257
3. PERFORATIONS IN CLOSING MEMBRANE OF PITS 258
V. SUMMARY 259

I. INTRODUCTION

The laws of refraction have an interest in microscopy, not merely in connection with the definition of anatomical details, but also in the determination of the chemical or physical qualities of any part or product of the cell. Such laws are of universal application, have the advantage of great precision, and serve to differentiate many characters not readily brought out by stains or chemical reagents. Furthermore, a knowledge of the refractive properties of the various tissues is indispensable if one is to obtain the clearest definition of tissue outlines by aid of the microscope. Current methods, however, go little further than listing the indices of refraction of media in which tissues may be mounted,

* Contribution from the Osborn Botanical Laboratory.

all of which have little significance in anatomical work unless the index of refraction of the tissue substances is also given. In as much as many, if not most, of the ultra-microscopic characters of cell membranes are to a greater or less extent associated with the refractive powers of these membranes, the laws of refraction have their special application in the investigation of the physical or chemical composition as well as in the anatomical characters of cell membranes. Such laws, therefore, in their application to plant tissues, come well within the scope of botanical research, and, as such, open a wide field for investigation, particularly when more than ordinary precision is required.

Owing, apparently, to chemical change, the refraction of a plant membrane is peculiarly liable to actual variation during growth; still more characteristic of the plant membrane, due to its hygroscopic qualities, are the apparent fluctuations (deviations) in refraction by virtue of which it absorbs (imbibes) water and other refractive liquids in which it may be immersed.

A study of the refraction of light in plant tissues and of some of the conditions affecting or apparently affecting its stability was carried on by the writer at intervals during the years 1917–1919, at the Osborn Botanical Laboratory. The writer expresses indebtedness to Professor A. W. Evans and other members of the Department of Botany and to the members of the School of Forestry, for every encouragement throughout the investigation; also, to Professor C. S. Hastings of the Department of Physics for many valuable suggestions, and for verification of the work with refractive liquids. The methods described constitute a supplement to the more general technique for woody tissues given in an earlier paper ('19, p. 138).

II. METHODS OF MEASURING REFRACTION WITH THE MICROSCOPE

The methods by which it is possible to determine the refraction of an object (under the microscope) depend on the use of mounting media of known refractive powers. With a gradient series of such media, the index of refraction of an object may be measured with accuracy to the second decimal place.

1. REFRACTIVE LIQUIDS

To measure the refraction of an object microscopically a gradient series of refractive liquids must first be prepared. For plant tissues in the dry condition, a series of twenty liquids differing from each other in refraction by about .005 and ranging from 1.499 to 1.598 was found generally satisfactory. Either aqueous solutions or oils may be used in making up such a series, both water and oil possessing certain advantages over the alternative solvent. Aqueous solutions, for instance, would probably be best for measuring the refractive powers of the living contents of a cell. But such solutions are absorbed by many tissues more than are oils. One in particular of the solutions, to be described presently, the McLean solution, causes great swelling of mucilaginous membranes, which, for certain purposes, is desirable; but unfortunately, it breaks down the structure of starch grains. Water solutions are open to the objection that they tend to evaporate, with the result that the concentration and refraction of the liquids are changed. For general purposes, therefore, non-volatile oil mixtures proved to be superior to aqueous solutions, and a series made up from mixtures of castor oil (n = 1.49) and clove oil (n = 1.535) for refractions below 1.535, and of clove oil and naphthalene α monobromated (n = 1.65) for the higher refractions gave excellent results (n indicating index of refraction). The mixtures and their respective indices of refraction are indicated in TABLE I.

A gradient series of refractive liquids may be prepared by mixing a liquid of high refraction with one of low refraction. When castor oil is mixed with clove oil, or clove oil with naphthalene α monobromated, the index of refraction of the mixture is proportional to the volumes of the liquids mixed. Or, if V_a and V_b are the volumes of the liquids mixed and n_a and n_b the respective indices of refraction, then

$$\frac{V_a(n_a - 1) + V_b(n_b - 1)}{V_a + V_b} = n - 1$$

If a series of mixtures is now prepared, in each of which mixtures the relative volume of one of the oils of known refraction is decreased by a constant quantity (e.g., 10, 9, 8, . . . 0) while the

TABLE I

A SERIES OF REFRACTIVE OILS USED IN THE DETERMINATION OF RELATIVE REFRACTION

The test liquids are made from mixtures of naphthalene α monobromated (naphth.) clove oil (clove), and castor oil (cast.), and are arranged in sequence according to refraction. O indicates in what media the outlines are invisible or nearly so; + or −, that the refraction is either more or less than that of the liquid.

Test liquids			Lignified and furfurol-reacting tissues							Pectin, cellulose tissues					Cell contents			
Vol.		Index of refraction	Pith, *Tilia*	Vessels, *Tilia*	Normal fibers, *Alphitonia* (secondary wall)	Mucilaginous fibers, *Alphitonia*		Bast fibers, *Tilia* (secondary wall)	Middle lamella, *Tilia* (primary wall)	Cambium, middle lamella, *Tilia*	Phloem (soft bast), *Tilia*	Collenchyma *Tilia* = R. membrane, *Pyrus*	Primary cortex, *Tilia*	Reserve cellulose, date seed	Starch, wheat	Aleurone, *Zea*	Calcium oxalate trihydrate	Cork, cuticle
Naphth.	Clove					Secondary layer	Mucilaginous layer											
11	9	1.598	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
10	10	1.592	−	−	−	−	−	−	O	−	−	−	−	−	−	−	−	−
9	11	1.587	−	−	−	−	−	−	O	−	−	−	−	−	−	−	−	−
8	12	1.581	−	−	−	−	−	−	+	−	−	−	−	−	−	O	−	−
7	13	1.575	−	−	−	−	−	−	+	−	−	−	−	−	−	O	−	−
6	14	1.569	−	−	−	−	−	−	+	−	−	−	−	−	−	O	−	−
5	15	1.564	−	−	−	−	−	−	+	−	−	−	−	−	−	O	−	−
4	16	1.558	−	−	−	−	O	−	+	−	−	O	−	−	−	O	−	−
3	17	1.552	−	O	O	−	O	−	+	−	−	O	O	−	−	+	+	−
2	18	1.545	−	O	O	−	O	−	+	O	O	O	O	−	−	+	+	−
1	19	1.540	−	O	O	−	O	−	+	O	O	O	O	O	O	+	+	−
0	10	1.535	O	O	O	O	O	−	+	O	O	O	O	O	O	+	+	−
Cast.																		
1	9	1.531	O	O	+	O	O	−	+	O	O	O	O	O	O	+	+	O
2	8	1.526	O	O	+	O	O	−	+	+	O	O	O	O	O	+	+	O
3	7	1.521	O	+	+	+	O	O	+	+	+	O	O	+	O	+	+	O
4	6	1.517	+	+	+	+	O	O	+	+	+	O	O	+	O	+	+	+
5	5	1.512	+	+	+	+	O	O	+	+	+	O	O	+	+	+	+	+
6	4	1.508	+	+	+	+	O	+	+	+	+	O	O	+	+	+	+	+
7	3	1.503	+	+	+	+	+	+	+	+	+	O	O	+	+	+	+	+
8	2	1.499	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

second is correspondingly increased (0, 1, 2, . . . 10), or in such a way that the ratio of volumes of the two liquids (in a series of eleven mixtures) would be to each other in the successive mixtures as $\frac{10}{0}$ $\frac{9}{1}$ $\frac{8}{2}$ $\frac{7}{3}$ . . . $\frac{0}{10}$, then the indices of refraction of the respective mixtures will form a gradient series (see TABLE I). In order to obtain any required difference in refraction between consecutive liquids in the series, it is only necessary to determine the constant by which the relative volumes are consecutively changed.

To measure the volumes, a medicine dropper may be used, but allowance must be made for the relative size of a drop in each

case. This may be done by measuring at room temperature (70° F.) the volume of 200 drops of each of the oils used in the mixture. By this means a drop of castor oil was found to have a volume equal to that of 1.8 drops of clove oil, and 1 drop of naphthalene α monobromated, 0.95 the volume of a drop of clove oil.

2. PREPARATION OF THE TISSUES FOR STUDY

In preparing tissues for the measurment of their refraction the following schedule was adopted.

1. Cut as many sections, 10–40 μ thick, as there are refractive liquids in the series, and wash in water for 10 minutes or longer.

2. Transfer each section to a separate slide and cover with a glass slip.

3. Dry for at least fifteen minutes at 100° C. An electric oven is useful for this purpose.

4. Remove the slides one at a time from the oven and mount each section in a different refractive liquid of the series. The samples of tissue are then ready for microscopic examination.

3. DETERMINATION OF RELATIVE REFRACTION

For this purpose, an apochromatic microscope with Abbé condenser and movable sub-stage diaphragm was used, but any microscope with the usual accessories will answer for ordinary purposes. A magnification of about 800 diameters was usually used in the examination. The refraction of the object relative to that of the liquid was determined in each case by one or more of the following methods.

A. By oblique illumination

The partly-closed sub-stage diaphragm is shifted to one side. If the refraction of the tissue mass is above that of the liquid, the outlines appear dark on the side toward which the diaphragm is shifted. But if the refraction of the tissue mass is below that of the liquid, the outlines appear dark on the side away from which the diaphragm is shifted. In TABLE I the refraction of the tissue or body examined is indicated as + (above) or − (below) with respect to the refractive index of the liquid in which it is immersed.

If the dispersion (i.e., the difference between the indices of refraction of light of long and of short wave-length) of the liquid is not the same as that of the object, it is possible to find a common index of refraction for light of one wave-length only. In this case, the refracted rays give fringes of color such as blue on one side and red on the other. McLean's solution, which has a high dispersion, causes wide color fringes with the reserve cellulose of date seed, in dilutions having a refraction between 1.531 and 1.535. This method is particularly useful in determining whether the refraction of an object is above or below that of the liquid.

B. By direct illumination

Relative brightness.—In studying tissues, the relative refraction of a transparent object is often indicated by the illumination of its transverse surface. On raising the tube of the microscope slightly from sharp focus, an object whose refractive index is higher than that of the tissues or liquid in contact with it appears relatively bright except at the border (see FIGS. 1 and 2). An object of lower refraction, on the other hand, appears relatively dark. On lowering the tube the relative brightness is reversed in each case.

The Becke line.—A few bodies, such as the crystals of calcium oxalate in the crystal-parenchyma of woody tissues, are visible in all liquids of the series. The refraction of such bodies is best determined as follows: An uncovered edge of the crystal is found, which projects into the liquid. When brought into sharp focus, a narrow band of bright light—the Becke line—defines the outline. On focusing upward, this band of light appears to move in the direction of the body of highest refraction, i.e., outward from the border into the liquid, if the refraction of the liquid is higher than that of the crystal, or inward from the border toward the centre of the crystal if the refraction of the crystal is higher than that of the liquid.

C. Modifications with hygroscopic tissues

In applying the methods above described to plant tissues, certain modifications are necessary, due to the fact that most membranes tend to imbibe the refractive liquids in which

they are immersed and to become invisible in two or more liquids of the series. This latter phenomenon is attributable to the fact that the refraction index of the mass, i.e., the dry tissue substance together with whatever of the refractive liquid may have been imbibed, here approaches that of the liquid in which it is mounted; and under these conditions it would be recorded as O in the tabulation (see TABLE I). In such cases the refractive power of the tissue substance is taken as the mean between the liquid of highest refraction and the one of lowest refraction in which the outlines are barely distinguishable. Further observations regarding the refraction of hygroscopic bodies are made below.

D. Relative accuracy of the methods

The accuracy with which the index of refraction of an object may be determined by the foregoing methods obviously depends, in the first place, upon the accuracy of the calculated indices of the liquid mixtures. In a test made of one of the liquid mixtures (castor oil, 5 volumes: clove oil, 5 volumes), for example, all the figures in the calculated index (1.512 +) were found to be correct; in fact, no errors were found in the calculated indices of any of the mixtures; but for extreme accuracy, the index of refraction of the liquid should be standardized as a matter of precaution.

With accurately standardized liquids, the index of refraction of minerals may, according to Wright ('11, p. 96), be determined with an exactness of ±.001 when monochromatic illumination (strong sodium light) is used, and Wright considers the method by oblique illumination about as accurate as the Becke line method. By the Becke line method the refraction of calcium oxalate trihydrate was found by the writer to lie between 1.568 and 1.552; and with a series of liquids intermediate in refraction between the ones used, it would doubtless have been possible to have made a more exact determination. The method employed to determine the refraction of hygroscopic bodies is less exact than methods applicable to crystalline bodies, but determinations may be carried to the hundredths with reasonable accuracy.

III. REFRACTION OF TISSUES

The results recorded in TABLE I bring out wide relative differences in refraction in different tissues or in different parts of

the same tissue; also, it is seen that the index of refraction of any given membrane may deviate widely from the average or may actually change during growth.

1. RELATIVE REFRACTION

As shown in TABLE I, there is no single index of refraction which is common to even the different membranes of a tissue. A fair average for dehydrated tissues would be 1.53, or .01 above that of Canada balsam or dammar (1.52); but the refraction of many bodies, particularly the cell contents, differs widely from the mean. For the dehydrated tissues examined by the writer, n (the index of refraction) varies from 1.50 to 1.59; but had protoplasm and other living bodies with high water content been included, far lower refractions would doubtless have been found, and the range in refraction would probably have been about three times as great.

2. DEVIATION IN REFRACTION WITH IMBIBITION

Certain crystalline bodies found in plant tissues, such as the crystals of calcium oxalate, do not become invisible in any of the refractive liquids: no physical change takes place when they are brought in contact with the oil mixtures and no deviation or change in refraction occurs. An organic membrane on the other hand, as already noted, imbibes the liquid in which it is mounted. As a result of the physical change which thus results in the mass of the membrane, the latter becomes invisible, or nearly so, in two or more consecutive liquids of the refractive series, namely, in those whose index of refraction approaches most closely that of the membrane after it has absorbed as much as it will of the refractive liquid in which it is mounted. In other words, the refraction of the membrane mass, after imbibition, deviates from that of the tissue substance according to the volume and refractive index of liquid taken up, the principle involved being nearly the same as that observed in preparing the series of refractive liquids.

Different membranes show great differences in the relative deviation of refraction. Cuticle, cork, true wood fibers, and middle lamella, for example, show relatively little deviation; collenchyma, primary cortex, and mucilaginous membranes, on the other hand,

take up relatively more of the liquid and show a correspondingly wide deviation in refraction.

The imbibition of liquid is also indicated by the swelling of the membrane, and those membranes which have the widest deviation in refraction, as, for example, the mucilaginous membranes in woody fibers, are the ones which swell the most. Not infrequently such membranes, as shown by an accurate projection published in a previous paper ('19, p. 143), may swell to almost double thickness when dehydrated sections are mounted in water, glycerine, or McLean's solution. But refraction is a more delicate index than swelling in that it serves to differentiate the particular portions of a membrane which take up the liquid.

Some extremely hygroscopic membranes are invisible, or nearly so, in liquids differing in refraction from one another by more than .055. There thus is an apparently close relation between imbibition and refraction. But since visibility of outline is partly dependent upon other factors, such as the relative light dispersion of the liquid and the object, the deviation in refraction may not be exactly proportional to the imbibition of liquid.

3. CHANGE IN REFRACTION WITH GROWTH

Deviations or apparent fluctuations in refraction, such as those above described, are not associated with any actual change in refraction of the membrane substance; on removing the imbibition liquid from the tissue, the refraction of the membrane is found unchanged. But, during growth, actual changes in refraction may occur, as, for example, in the middle lamella of wood cells. In the early stages of cambial growth, such as may be found in the stems of woody angiosperms, the membrane which subsequently becomes the middle lamella has a refractive index of about 1.53, or the average for plant tissues. In subsequent stages, such as occur in the narrow zone of undifferentiated tissue next to the true cambium, where the thin-walled immature cells are in various stages of elongation, the refraction of this membrane increases with progressive differentiation. Thus, as soon as growth in length of the wood cells has ceased and the walls begin to thicken prominently by apposition—the stage in which the first secondary thickenings of the fibers appear and the tissue

hardens—there is a rapid rise in the index of refraction of the middle lamella to 1.59, or .135 above that of glycerine (FIG. 1), at which it remains nearly constant. Such a change in refraction can hardly be explained except in relation to a change in the chemical composition of the pectin lamella, as a result of which, beginning as a membrane of average refraction, it quickly becomes the most refractive of all.

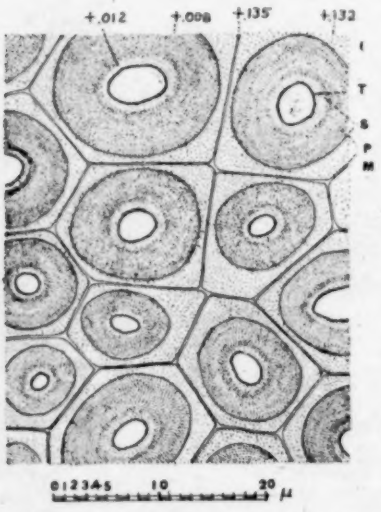


FIG. 1. Abbé camera drawing of fibers of Ipê tabaco (*Tecoma* sp.), mounted in glycerine ($n = 1.46$). All membranes are + with reference to the glycerine medium. In upper sharp focus, the refractive membranes appear bright; this is indicated in the shading. The middle lamella (*M*) is less distinct in glycerine than indicated in the drawing. The membranes (*M*, middle lamella, and *P*, primary thickening of the fiber) are highly refractive ($n = 1.59$) and appear brightly illuminated, especially the middle lamella. The secondary layer (*S*) is less refractive ($n = 1.54$) and relatively dark. *T*, tertiary thickening.

The primary thickening of wood fibers (*P*, FIG. 1) is also composed of a highly refractive material. In xerophytic species of *Tecoma* and a number of other woods, this membrane first appears as a collenchyma-like thickening upon the sides of the middle lamella, in cells which lie near the true cambium, and which have not ceased to elongate and to glide upon one another. This membrane, though much thickened in species of *Tecoma* and certain other genera, is ordinarily thin and, because of similarity in the refractive and staining qualities, is likely to be mistaken as part of a somewhat thickened middle lamella. Both membranes stain deeply with haematoxylin, methylene blue, fuchsin, and other stains for pectic bodies, and together constitute by far the most refractive (.132 –.135 above glycerine) membranes in the mature xylem. But the refraction of the primary wall is slightly lower (about .003) than that of the middle lamella, and, when observed in a refractive medium at which the former becomes invisible (about 1.545 for

Tecoma), the true middle lamella is nearly as sharply defined as in collenchyma (C, FIG. 2).

The observed differences in refraction here are probably associated with differences in mineral content of the membrane studied. Of the mineral elements which enter into the composition of woody tissues, calcium, especially in the form of calcium carbonate, would seem to be the only one likely to bring about an increase in refraction as great as that noted in the middle lamella and primary wall. The presence of silica, for example, in portions of the secondary layer (FIG. 1, *S*) is associated with a much lower refractive index.* The rise in refraction of the middle lamella and primary wall during development finds its most reasonable explanation in the supposition that in these pectic membranes the relative proportion of calcium is greatly increased as soon as the cells have attained their mature size and the mechanical tissues begin to acquire hardness and strength. Apparently, these membranes acquire their mechanical rigidity as a result, in part at least, of the addition of calcium, possibly in combined form, to the pectin; and it is of interest to add that both by chemical treatment and by the action of certain enzymes, as will be described presently, the primary wall may be softened and the middle lamella reduced to a jelly-like consistency, so that cells glide upon one another as during development; even closing membranes of

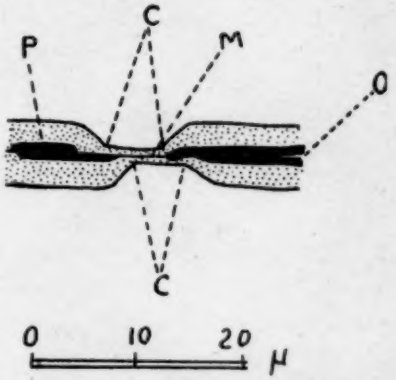


FIG. 2. Longitudinal section of a wall between two thick-walled parenchyma cells in the stem of *Dracaena aurea*, after twenty-four hours treatment in Easter lily pollen enzyme solution. The middle lamella (*M*) has softened, allowing the cells to glide upon another, the closing membrane (*C*) of a simple pit has separated longitudinally along the middle lamella and one part has glided upon the other. In places, the primary wall (*P*) is shown stained deeply with haematoxylin and at *O* it has separated along the middle lamella.

* The presence of highly silicified longitudinal tracts in the secondary layer of wood fibers of *Tecoma* has been demonstrated by the writer and will be published in a subsequent paper on fiber skeletons.

pits may be split in a manner similar to that observed by Neeff in growing fibers of wood ('14, p. 503) and bast ('14, p. 510).

The treatment by which the middle lamella was softened is as follows: thin sections (3 μ thick) of the wood of *Dracaena* were placed for twenty-four hours in a 25 per cent alcoholic solution of hydrochloric acid, then for twelve hours in 20 per cent ammonia. After such treatment the primary wall and particularly the middle lamella were etched and softened to a degree that permitted cells to glide upon one another, the closing membranes of pits were divided along the middle lamella, so that one part moved upon another. By this treatment with acid alcohol, according to Mangin (Haas and Hill, '13, p. 127), the combined pectin is freed from its bases (e.g. calcium) and is then dissolved by the ammonia. It is of interest to note that an effect similar to that obtained by hydrochloric acid and ammonia (FIG. 2) was obtained by placing sections, 2.5 μ thick, for twenty-four hours upon wet Easter lily pollen moistened with water, the membranes, in this case, being acted upon by an enzyme. In sections of *Tecoma*, the middle lamella and primary walls were similarly etched and softened.

Not only the middle lamella and primary wall but also all other tissue elements have, with possibly a few exceptions, a characteristic refractive index; it is remarkable to what an extent the various membranes become differentiated in their refractive properties during growth. As will be shown presently, a membrane which can hardly be differentiated by reagents may be clearly defined by its refractive properties.

IV. DEFINITION OF ANATOMICAL DETAILS

Success in obtaining clear microscopic definition is in no small measure dependent upon the refraction of light by the mounting medium. It is of the utmost importance that the index of refraction of the tissue should bear a definite relation to that of the medium in which it is mounted.

1. REFRACTION OF MOUNTING MEDIA

When the refractive properties of a tissue are known, definite refractive effects may be obtained by selecting media having the necessary indices of refraction. The following list (TABLE II) includes some of the refractive media most useful for this purpose:

TABLE II

REFRACTION OF MEDIA

	Index of refraction
1. Water	1.336
2. Alcohol	1.37
3. Fluorite	1.433
4. Glycerine jelly	1.44
5. Glycerine	1.46–1.47
6. Castor oil	1.49
7. Xylol	1.50
8. Oil of red cedar (*Juniperus procera*)	1.512
9. Crown glass	1.518
10. Dammar; Canada balsam	1.52
11. Clove oil	1.535
12. Quartz	1.544
13. Styrax	1.58
14. McLean's solution; flint glass	1.621
15. Balsam of Tolu	1.628
16. Naphthalene α monobromated	1.65
17. Iceland spar (calcium carbonate) (Hastings '88)	1.658 and 1.48
18. Tolu dissolved in naphthalene α monobromated	1.72
19. Quinidine	1.8?

A few comments on some of the various media listed may be made:

Water; alcohol.—These media have an index of refraction far below (a difference of about −0.20) that of the average for plant tissues. If the section is thin, these media have no superior for the definition of minute details in tissues which shrink on dehydration. But, on the other hand, a section of ordinary thickness (20–70 μ) lacks transparency in these media and the outlines are confused. In such sections, greater transparency and therefore clearer definition may be obtained by mounting in some medium such as xylol, whose index of refraction differs but little (about 0.02) from that of the tissue.

Glycerine; glycerine jelly.—Unstained sections of stem tissues, from which air has been removed by standing in alcohol, mounted in glycerine and permanently cemented with Brunswick black or gold size, require little time to prepare and are frequently superior to stained sections for observing minute details such as the pitting of fibers or vessels, as well as the broader details of outline. Glycerine jelly is particularly to be recommended for unstained sections of woody tissues.

Dammar; Canada balsam.—It is unfortunate that the refractive index of both dammar and Canada balsam approach too closely those of the stem tissues to allow their use for mounting unstained sections of any of the hygroscopic tissues of the stem. Details in some of the soft tissues of the bark, as will be shown presently, are also obscured through shrinkage during the process of dehydration. These media have a refractive index about .01 below the average for dehydrated plant tissues and, for general purposes, are fully deserving of the esteem in which they are held as media for mounting stained sections.

McLean's solution.—This solution, suggested by McLean ('14), is prepared by dissolving in a saturated aqueous solution of potassium iodide as much mercury biniodide as it will take up. This solution has a high index of refraction (1.6210 for solution of full strength), equal to that of flint glass and far exceeding that of any of the tissues examined; it is readily diluted with water, the index of refraction of which is lower than that of any constituent of plant tissue. This liquid may therefore be regarded as one of the best for preparing a series of aqueous dilutions for measuring refraction in tissues, and the principle involved in diluting with water is the same as that earlier given and adopted in mixing the oils of a refraction series. By such a series of liquids a wide range in refraction (1.33–1.62) may be covered, and if successive dilutions were made to differ from each other by $n = .005$, there would be 59 liquids in this series. As before mentioned, tissues imbibe aqueous solutions more readily than the oils, causing wider deviations in refraction, but the mean refractions of tissues determined by their use were the same as those determined by oils. McLean's solution also has high dispersion or, in other words, a wide difference between the index of refraction for light of long wave-length (red) and light of short wave-length (blue).

As a medium for mounting soft tissues, this solution possesses the advantage of not requiring dehydration, so that such tissues may be mounted directly without danger of shrinkage. But it has the serious disadvantage of causing wide deviation of refraction in certain tissues, such as collenchyma, as a result of which the outlines may become quite invisible.

Tolu.—This oleo-resin (balsam), obtained from the bark of a leguminous tree, *Myroxylon toluifera* H. B. K., of South America, brings out satisfactorily the pitting and other details of unstained macerated preparations.

Naphthalene α monobromated.—If sections are thin, naphthalene α monobromated defines clearly all tissues of the stem. Where dehydration can be accomplished without too great shrinkage of the tissue, details such as the pits in hypodermal collenchyma appear very distinctly. This oil is also one of the best for use in measuring refraction of tissues by the microscopic method.

Quinidine.—This is perhaps the most satisfactory medium of all for the definition of minute details. Also, all tissues of the bark appear clear and well defined except for details affected by shrinkage or high temperature. In using this organic substance, it is liquified on the slide at a temperature above that of boiling water. Professor Hastings states that quinidine crystallizes or spoils with time and, further, that highly refractive media, such as quinidine, tolu, and styrax, define with great clearness the minute details of diatom cysts.

2. PITS AND MIDDLE LAMELLA IN COLLENCHYMA

The part played by refraction in bringing out details may be demonstrated by comparing cross-sections of the collenchyma of some woody stem, such as *Pyrus communis* L. or *Aucuba japonica* Thunbg., mounted in Canada balsam with sections mounted in other media. In balsam mounts the prominent system of pits connecting the cells is usually obscured (FIG. 3, *A*), whether stained or not, and it is worthy of note, in this connection, that the presence of such pits in the collenchyma of woody stems, though a characteristic feature of this tissue in nearly all dicotyledonous trees and in many other woody plants, has seldom been noted in anatomical drawings or descriptions, even in such standard texts as that of Moeller ('82), although it can be readily seen when in sections which have been mounted in suitable media (FIG. 3, *B*). Thus, this pitting system becomes very clear in naphthalene α monobromated, invisible or nearly so in potassium iodide-mercury biniodide, and visible again in glycerine (FIG. 3 *B*) or water. The middle lamella, however, is not clearly defined in any of these

media; but if the section is mounted in a medium whose index of refraction (about 1.526) approaches sufficiently close to that of the inner and outer thickenings (*I* and *O*, FIG. 3), then the middle lamella, whose index of refraction is higher (+) than this, stands out plainly (as indicated in FIG. 3, *C*) and is the only portion of the wall which can be seen even under the most favorable conditions of illumination. In alcohol and quinidine, dehydration may cause shrinkage of the tissues to such an extent that not as good results are obtained as would be expected from the refractive

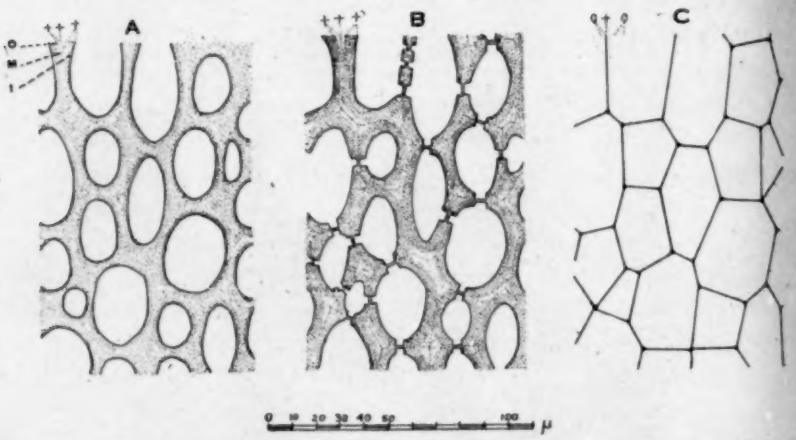


FIG. 3. Appearance of a single section of collenchyma of *Pyrus communis* L. mounted in three different refractive media. *A*, in Canada balsam, $n = 1.52$; there is little or no definition of minute detail. *B*, the same section as *A*, but mounted in glycerine, $n = 1.46$; the pits are clearly defined. *C*, again the same section, but mounted in a mixture of castor oil, 2 volumes and clove oil 8 volumes, $n = 1.526$; in a medium of this refraction, the middle lamella (*M*) is clearly defined, but the other membranes (*I* and *O*) are invisible. The tube is slightly raised from sharp focus; the inner thickening (*I*) of the collenchyma walls is less refractive than the outer thickening (*O*) and appears less brightly illuminated in *A* and *B*.

properties of these media. Glycerine, on the other hand, causes a slight swelling of the walls, but this is of advantage, in this case, in bringing out the details sought.

3. PERFORATIONS IN CLOSING MEMBRANE OF PITS

The minute perforations through the closing membranes of pits, as in case of the bordered pits of conifers, can be readily demonstrated in the following manner: place a few drops of a

dilute solution of xylol dammar (mounting consistency diluted about twenty times) on the transverse face of the dry woody tissue to be tested. Section immediately to 10 μ and mount in glycerine. The perforations (FIG. 4) each with a small globule of dammar in or attached to it, are then easily visible in the glycerine medium. The cover glass may be cemented with gold size, after the manner of glycerine permanent mounts, and the preparation will keep for at least several weeks, often improving on standing a few days; but the slide should always be kept face upward.

In mounting tissues in highly refractive media, care should be taken to press the cover slip down tightly against the tissue, to exclude any excess liquid from above the section. With quinidine, for example, an effect of chromatic aberration may be produced by a film of the liquid between the lignified membranes of the section and the cover slip.

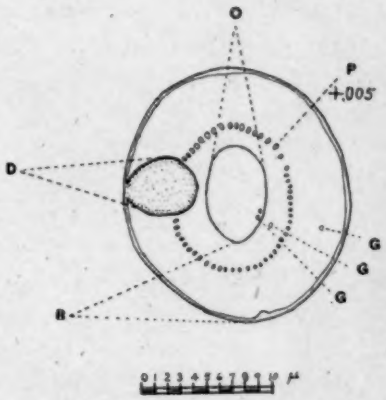


FIG. 4. Bordered pit of sugar pine, *Pinus Lambertiana*, after treatment with dammar to bring out perforations; *D*, a large globule of dammar floating partially free in the glycerine within the border (*B*); *P*, perforations through the closing membrane, arranged in a circle at the edge of the torus and visible through the transparent border; *G*, globules of dammar detached from perforation at edge of torus and floating free in the glycerine; *O*, orifice.

V. SUMMARY

1. In the fully developed tissues examined, the refractive index of the material of which any given cell-membrane or cell-content is composed is fairly constant with uniform conditions of temperature; but during the growth of a tissue the refraction of its constituent materials may change enormously. Such changes in refraction are evidently associated with corresponding changes in the chemical composition.

2. The indices of refraction of the various membranes and other elements, of which mature plant tissues are composed, differ greatly from one another, and a given tissue complex will

rarely if ever have a single refractive index. A fair average, however, would be about 1.53, or .01 above that of Canada balsam.

3. Most tissues readily absorb water and other refractive liquids, in differing amount according to the hygroscopic qualities of the tissue. This causes the refraction of the mass (tissue substance + imbibition liquid) to deviate correspondingly from that of the tissue substance. The refractive index of the membrane mass is highly sensitive to physical changes of this kind.

4. The laws of refraction have their special application to the investigation of both the anatomical characters and the physical and chemical properties of plant tissues.

5. With average sections, clearer definition is obtained when the index of refraction of the medium is below, rather than above, that of the tissue substance. A difference of at least .05 between tissue mass and the medium in which it is mounted is desirable to obtain clear definition of small perforations and similar minute details, and highly refractive media such as styrax, tolu, and quinidine bring out such details with the greatest possible clearness; in moderately thin sections, a difference of .005 is sufficient for the definition of general outlines, and may give clearer definition for general features than a greater difference in refraction.

6. In selecting a medium, to obtain a given difference in refraction between it and the tissue mass, due allowance must be made for the deviation in refraction brought about by the imbibition of liquid by the tissue.

YALE UNIVERSITY

LITERATURE CITED

Brown, F. B. H. '19. The preparation and treatment of woods for microscopic study. Bull. Torrey Club **46**: 127–150. *f. 1–6.*

Hastings, C. S. '88. On the law of double refraction in Iceland spar. Amer. Jour. Sci. **35**: 60–73.

McLean, R. C. '14. A new method of staining Cyanophyceae. New Phytol. **13**: 71–72.

Moeller, J. '82. Anatomie der Baumrinden. Berlin.

Neeff, F. '14. Uber Zellumlagerung—Ein Beitrag zur experimentellen Anatomie. Zeits. Bot. **6**: 465–546. *pl. 2 + f. 1–32.*

Wright, F. E. '11. The methods of petrographic-microscopic research, their relative accuracy and range of application. Publ. Carnegie Inst. Washington, No. 158.

The physical factors in the growth of the tomato

D. T. MacDougal

The tissues of animals and of the greater number of plants accumulate carbohydrates, proteins, salts and other solids during the course of growth in such manner that the relative dry weight of an organ is least in the embryonic or earlier stages, and increases progressively so that the proportion of solid material is highest and of water is lowest at maturity.

The stems and leaves of succulents and such berry-like fruits as the tomato (*Lycopersicum*) have been found to reverse this relation,* and melons, mushrooms and similar structures probably do the same.

Tomatoes 14–18 mm. in diameter grown at the Coastal Laboratory, Carmel, California, were found to contain but 87 per cent of water, while mature fruits consisted of 91 per cent water and 9 per cent solid material. An analysis by Albahary gave 93.63 per cent of water in young tomatoes and 94.3 per cent in ripe fruits.†

In addition to the measurements already published, it seems desirable to give the data secured in 1919, to make further comparisons between the apparent growth as found by the increase in the diameter of the fruits, and to record the actual increase in volume,‡ and to formulate an explanation of growth based upon the osmotic and colloidal action of the plasmatic substances under the influence of cell-sap of varying composition.

Vigorous plants bearing fruits in all stages were available in August and September, 1919, and suitable stages were erected

* MacDougal, D. T. Hydration and growth. Publ. Carnegie Inst. Washington, No. 297, 1920. See especially pages 166–172. Also, Hydration and growth, Proc. Amer. Phil. Soc. **58**: 346–373. 1919.

† Albahary, F. M. Etude chimique de la maturation du *Lycopersicum esculentum* (Tomate). Compt. Rend. Acad. Sci. Paris, **147**: 146–147. 1908.

‡ Note. In a discussion of this subject in the Proc. Amer. Phil. Soc. **58**: 367, 1911, the formula for obtaining the volume of a sphere, 4/3 (PiR^3) was erroneously given as (PiR^2).

so that small green fruits were resting securely on cork blocks. The vertical swinging arm of an auxograph also tipped with cork was now brought to rest on the upper surface of the fruit. Any variation in volume would be denoted by a deviation of the pen tracing from a horizontal line. A full description of this apparatus is given in connection with *f. 1* of the publication cited above.

The temperatures in this and in my other work on growth and swelling were taken by mercurial thermometers thrust into tissues or in contact with the bodies under observation. In the present instance small thermometers of the "clinical" type were thrust into fruits similar to those being measured and remained there during their development, causing but little divergence from normal morphology. The fruit stood between 15 and 20° C. during the experiments, which was on an average 8–10° below the optimum for these plants, but the relative humidity was favorably high.

The points to be presented may be demonstrated by the action of two fruits which were kept under continuous measurement, one for thirty-eight and the other for thirty-five days.

Fruit No. 1 had a diameter of 6 mm. and a volume of 113 cu. mm. when its measurement was begun, and its volume increased at the rate of 130 cu. mm. daily for six days, 402 cu. mm. for the following seven days, 409 cu. mm. for seven days, 560 cu. mm. for nine days and 930 cu. mm. for the last 9 days, at which time the total volume was 19,864 cu. mm.

The rate of increase of the diameter of these fruits during the same periods was 0.8 mm., 1 mm., 0.55 mm. and 0.6 mm. daily. A graph plotted from these daily rates would give a highly erroneous impression as to the actual course of accession of material to the fruits, which is the essential feature of growth. If the rate of growth be multiplied by twenty-five and placed in a table above the average daily increase in volume for the same period, a comparison may be made without the aid of a graph.

It is to be seen that the rate of increase in the diameter of the

Rate of increase	First week	Second week	Third week	Fourth week	Fifth week
Diameter	20	25	14	15	16
Volume	22	57	58	63	103

fruits at the maximum was not double the lowest rate, the highest occuring while the fruit was very small. Increase in volume, on the other hand, was progressively greater and as the fruit neared maturity water and material were being brought in nearly five times as fast as in young fruits. This delayed maximum might be connected with the low temperatures under which the development took place, as the highest rate of accession of material to fruits in 1918 at higher temperatures came somewhat earlier, although subsequent to the maximum increase in diameter.

A second fruit measured for thirty-five days was 8 mm. in axial diameter at the beginning and had a volume of 268 cu. mm. It increased 0.9 mm. in diameter and 167 cu. mm. daily for a week, 0.9 mm. daily for the second week with an accretion of 492 cu. mm., 0.7 mm. daily with an accretion of 412 cu. mm. in the third week, a rate of 0.67 mm. daily with an accretion of 1045 cu. mm. in the fourth week, and 0.3 mm. daily with an accretion of 600 cu. mm. in the last week.

A comparison of increases in diameter and volume is given below:

Rate of increase	First week	Second week	Third week	Fourth week	Fifth week
Diameter	27	27	21	20	9
Volume	27	56	59	150	185

The increase in diameter was at a rate which did not vary widely until at the last, when it dropped to less than half that which had previously prevailed. The rate of increase in volume rose steadily until at the end it was seven times as great as during the first week. The accretion is visualized as forming a layer on a globe, the volume of the fruit at any time being determined by the use of the formula $V = 4/3\ (PiR^3)$. A layer of a given thickness on a large fruit would obviously include a much greater amount of material than a layer of similar thickness on a small fruit.

The mechanical features of the growth of a storage body, as a fruit must be considered, are something different from those which prevail in a stem with its successively developing members or internodes, or in leaves which proceed to a definite average size

on maturity. In both types of growth the rate depends upon, or is influenced by, the amount of growth that has previously taken place, as has been demonstrated by Reed in his measurements of growth of the stems of apricot trees.

The stems of the tomato are enlarging during the growth of the fruits, the leaf surfaces are increasing and the pedicels of the fruits show an increasing cross section, so that not only is an additional amount of water and material available but its translocation may be facilitated.

It would be erroneous however to consider the fruits as reservoirs which passively receive the solutions poured into them through the stems. The fruits are in fact largely made up of rapidly enlarging masses of thin-walled cells which control absorption of material as occurs in living tissues. Liquids are drawn into these cells, not forced.

The forces which operate to carry water and solid material into a fruit may be grouped under osmosis and imbibition.

The freely soluble sugars which constitute about 9 per cent of the dry weight of young fruits and 38 per cent of ripe fruits, according to the analyses of Albahary, would operate to set up and maintain a turgidity that would probably reach eight to twelve atmospheres, and the attractive force of these substances would be one of the main factors in drawing material into the fruits.

It is well known, however, that fruits may withdraw water from stems that are relatively drier, and that roots may take up water from soils in which the osmotic action would be greater. To account for such action we must look to imbibition, the phenomena by which colloidal matter, such as jelly or wood, absorbs water or solutions and swells as a consequence of the hydration or addition of molecules of water to the aggregates of molecules of solid matter in their intimate structure, for growth under such circumstances.

Extensive investigations at the Desert Laboratory show that the living matter of plants is a mixture of albuminous substances and of pentosans or mucilages. Furthermore, parallel experiments with mixtures made in the laboratory show that in taking up water the plant behaves like a sac containing albumin and mucilages. It is necessary for a clear understanding of the action of the

plasmatic mass to realize that the albumin and mucilage may be taken to be in an interwoven meshwork, or if in suspension in separate globules.

This being the case, one may take up water and swell, or lose water and shrink, while the reverse action takes place in the other, which might also remain inactive. Thus the albumins swell most in acids or under the influence of free hydrogen ions, although being amphoteric they may also swell in hydroxyl ions or under the action of bases, while they are not very active in solutions of amino-compounds. The mucilages on the other hand are weak acids, swelling but little in acids, more under the action of hydroxyl ions or bases, still more in water, and reaching a maximum in amino-compounds.

Mixtures of mucilages and of albumins will, in the main, show reactions determined by the element which makes up the largest proportion of its mass. It follows therefore that the growing cell-masses of plants show swelling, hydration and growth reactions determined by the mucilages or pentosans, as modified by the albumins, acids, and salts present.

When we take up the facts disclosed by chemical analyses of tomatoes, we discover five things which must be taken into account in any attempt to make a physical-chemical explanation of growth These are as follows: (1) the proportion of sugar, including the mucilages, in the dry material increases from 9 to 37 per cent in the stage of enlargement including the formation of the seeds; (2) the acids, which include malic, phosphoric and citric, increase toward maturity; (3) the albumins decrease with development; (4) the ash or metallic bases increase from 4.5 to 10.75 per cent of the dry weight; and (5) the proportion of cellulose lessens as the fruit proceeds toward maturity.

If due weight be given to these factors or agencies the procedure in growth may be determined. Thus in the earlier stages the total albumins constitute less than 3 per cent of the dry weight and the sugars over 10 per cent, of which at least one third may be taken to be pentosans or mucilages. The colloidal mixture of equal parts of the two—mucilages and albumins—might be capable of showing a hydration capacity of over 3,000 per cent in a cell sap containing any one of a number of amino-acids which are

invariably present in cell-masses of this kind. The hydrogen ion concentration and the proportion of salts or bases is still low and their effect on the swelling would be limited. It would therefore be possible for these young fruits to make a notable amount of growth or expansion upon imbibition by the protein-pentosan plasma in a juice relatively low in salts and in acids.

Development and increase in size would however be attended by a lessened albumin content, making the plasma react more like agar in which hydration is lessened by salts and by acids, and imbibitional swelling would be notably decreased.

The accumulation of sugars in the cells occurring concurrently with lessened imbibition serves to set up an osmotic mechanism by which an increasing turgidity would result, serving as a distentive agency in the later stages of growth more than replacing the waning capacity for imbibition, and the action of osmosis in these fruits might have a heightened effect by reason of the fact that the cell walls of much of the fruit become "pectinated" and the proportion of cellulose becomes less, according to the analytical results of Albahary. This last named feature is the reverse of that in stems or vegetative tissues in which the cell walls become heavier and more indurated with progressive development.

The above conditions in the fruits are fully sustained by the following set of swelling reactions, which are entirely characteristic of a pentosan-protein colloid high in mucilages of the first group.

SWELLING OF RADIAL SLICES OF GROWING FRUITS 25–35 MM. IN DIAMETER

	Propionic acid	Alanine	Phenyl-alanine	Glycocoll	Water
0.01M	10%	25%	25%	25%	21%

In a later stage of the fruits when the skin is turning yellow, the salt content amounts to 10 per cent of the dry weight and the acidity is such that the sap has hydrogen ion concentration of a PH value = 4, while the albumins have decreased. The results of a set of swellings are as follows:*

	Propionic acid	Alanine	Phenyl-alanine	Glycocoll	Water
0.01M	16	15.5	11	15	15

* Cohn, E. J., J. Gross & O. Johnson. The isoelectric points of the proteins in certain vegetable juices. Jour. Gen. Physiol. 2: 145–160. 1919.

Regardless of other causes the acidity, high salt content and low albumin content would be sufficient to restrict absorption of water by the plasmatic colloids to two thirds the capacity shown by young fruits.

If we now turn to cultural experiments, ample confirmation is found for the conclusion that the amino-compounds which increase hydration or water absorption also facilitate or accelerate growth as measured in terms of dry weight, as found by Dachnowski and Gormley, Borowicov, Long, and Schreiner, Skinner and Beattie.*

Still one more feature of growth remains to be considered, that of certain retarding factors. The material which goes to increase the fruit of the tomato is about 90 per cent water. As water is being constantly transpired from the thin skins of these fruits, it is obvious that a hightened transpiration might throw off water at a rate which would use much of the liquid brought in by absorption and thus decrease growth. This occurs almost every day of sunshine and may result in the fruit having a lesser diameter at noon than at sunrise. Such cessation of growth in length and thickness of stems has been observed many times, and was earlier supposed to be due to the retarding action of light. It is however simply a loss of water greater than the absorption during the same time with a consequent shrinkage.

The principal conclusions supported by the foregoing may be briefly stated as follows:

1. The fruits of the tomato (*Lycopersicum*) furnish examples of development and growth without increase of dry weight.

---

* See MacDougal, D. T. Hydration and growth. Carnegie Inst. Washington Publ. No. 297, 1920. See pp. 51 and 52, 63 and 64, and the following titles not considered in the preparation of that publication:

Schreiner, O., J. J. Skinner. Specific action of organic compounds in modifying plant characteristics; methyl glycocoll versus glycocoll. Bot. Gaz. **59**: 445–463. 1915.

Skinner, J. J., & J. H. Beattie. Effect of asparagin on absorption and growth in wheat. Bull. Torrey Club **39**: 429–437. *pl. 33.* 1912. (Good account of previous work with asparagin.)

Shreiner, O., & J. J. Skinner. Experimental study of the effect of some nitrogenous soil constituents on growth. Nucleic acid and its decomposition products. Plant World **16**: 45–60. 1913.

Borowicov, G. A. On the action of different substances on the velocity of growth of vegetables. Publ. Soc. Nat. New Russia **41**: 15–194. 1916.

2. The rate of increase in diameter of such globose, berry-like fruits is not a correct or even approximate measure of actual growth considered as an accretion of water and solid material.

3. The time at which the greatest increase in diameter takes place may coincide with the greatest growth as exemplified in previously described observations, but the increase in thickness is not a direct index of growth in such bodies. Actual growth varies as the cube of the radius.

4. The culmination of the rate may not be reached until the fruit is in a stage approaching maturity. The maximum accretion generally takes place in a stage subsequent to the highest rate of increase of the diameter.

5. The internal factors which determine the rate and amount of growth of the tomato include the soluble sugars and the salts or bases which increase toward maturity, and the albumins and celluloses which decrease with development, while the amino-acids, not determined, probably do not vary so widely as to affect their value as growth accelerators.

6. The conjunction of low acidity and low salt content and sugar content would give a set of conditions for high imbibitional swelling of a pentosan-protein plasma in the earlier stages of growth which would be capable of carrying the fruit to an enlargement of 3,000 to 4,000 per cent of the dry matter, as determined by previous experiments in the hydration of such colloids. Otherwise expressed, imbibition would be capable of making a colloidal body like a fruit which would consist of 97.5 per cent water and 2.5 per cent solid matter.

7. The higher salt content and acidity of older fruits would operate to lessen imbibition in the fruits, which in this stage would be high in carbohydrates.

8. The above facts support the conclusion that the distentive force in growth of young fruits is chiefly imbibition. Osmotic action may play the more important part in later stages.

9. The growth of a fruit therefore is a resultant of two groups of activities, one ordinarily classed as imbibitional and the other associated with osmosis and turgidity.

10. Young fruits include 1 to 4 per cent more solid material than mature ones, these bodies being representative of a type of

plant structure in which the dry weight does not increase with age.

11. The amino-acids induce a greater swelling or absorption of water by the cell masses of growing tomato fruits than takes place in weak acid solutions or in water. This fact is in agreement with, and is probably fundamental to, the accelerating effect of these substances on growth.

12. Continuous measurements of tomato fruits reveal slackened growth or shrinkage in the midday period corresponding to the time of greatest transpiration, and it is concluded that water absorption during this period is balanced by the loss from the surface, in accordance with the behavior of many other structures, such as trunks and twigs of trees, stems of sunflowers, joints of *Opuntia*, and leaves of *Mesembryanthemum*.

DESERT LABORATORY

INDEX TO AMERICAN BOTANICAL LITERATURE

1914–1919

The aim of this Index is to include all current botanical literature written by Americans, published in America, or based upon American material; the word America being used in the broadest sense.

Reviews, and papers that relate exclusively to forestry, agriculture, horticulture, manufactured products of vegetable origin, or laboratory methods are not included, and no attempt is made to index the literature of bacteriology. An occasional exception is made in favor of some paper appearing in an American periodical which is devoted wholly to botany. Reprints are not mentioned unless they differ from the original in some important particular. If users of the Index will call the attention of the editor to errors or omissions, their kindness will be appreciated.

This Index is reprinted monthly on cards, and furnished in this form to subscribers at the rate of two cents for each card. Selections of cards are not permitted; each subscriber must take all cards published during the term of his subscription. Correspondence relating to the card issue should be addressed to the Treasurer of the Torrey Botanical Club.

Adams, J. F. The alternate stage of *Pucciniastrum Hydrangeae*. Mycologia **12**: 33–35. 1920.
Peridermium Hydrangeae(Berk. & Curt.) comb. nov.

Anthony, S. & Harlan, H. V. Germination of barley pollen. Jour. Agr. Research **18**: 525–536. *pl. 60–61 + f. 1–2.* 16 F 1920.

Appel, O., & Westerdijk, J. Die Gruppierung der durch Pilze hervorgerufenen Pflanzenkrankheiten. Zeits. Pflanzenkrank. **29**: 176–186. 1919.

Arthur, J. C. Two destructive rusts ready to invade the United States. Science II. **61**: 246–247. 5 Mr 1920.

Bergner, Dr. Singular phenomena in the kingdom of plants. Sci. Am. Monthly **1**: 121–123. *f. 1–9.* F 1920.

Bisby, G. R. Short cycle *Uromyces* of North America. Bot. Gaz. **69**: 193–217. *pl. 10.* 16 Mr 1920.

Boyer, C. S. Rare species of North American Diatomaceae. Bull. Torrey Club **47**: 57–71. *pl. 2.* 10 Mr 1920.

Bradshaw, R. V. Color variations of flowers. Am. Bot. **26**: 23–24. 20 F 1920.

Bradshaw, R. V. Rare plants of Oregon. Am. Bot. **26**: 18–19. 20 F 1920.
Erodium moschatum, Linaria Elatine, Centaurea nigra, Cynosurus echinatus.

Brown, N. A., & Harvey, R. B. Heart rot, rib rot and leaf spot of Chinese cabbages. Phytopathology **10**: 81–90. *f. 1–4.* 1920.

Brown, W. H., & Fischer, A. F. Philippine bamboos. Bull. Bur For. Philipp. Is. **15**: 9–32. *pl. 1–33.* 1918.

Brown, W. H., & Fischer, A. F. Philippine mangrove swamps. Bull. Bur. For. Philipp. Is. **17**: 11–127. *pl. 1–47.* 1918.

Burt, E. A. The Thelephoraceae of North America XI. Ann. Mo. Bot. Gard. **6**: 253–280. *pl.* 5. + *f. 1–15.* 2 Mr 1920.
Epithele sulphurea and *Lachnocladium erectum*, sp. nov.

Clinton, G. P. Infection experiments of *Pinus Strobus* with *Cronartium ribicola*. Bull. Conn. Agr. Exper. Sta. 214: 428–459. *pl. 37–44.* S 1919.

Clinton, G. P. Inspection of phaenogamic herbaria for rusts on *Ribes* sps. Bull. Conn. Agr. Exper. Sta. 214: 423–427. S 1919.

Clute, W. N. An unknown honeysuckle. Am. Bot. **26**: 17–18. 20 F 1920.

Cushman, L. B. *Aegopodium Podagraria variegata.* Am. Bot. **26**: 13–14. 20 F 1920.

Davidson, A. *Draba saxosa* n. sp. Bull. S. Calif. Acad. Sci. **19**: 11–12. Ja 1920.

Davidson, A. *Linanthus saxiphilus* n. sp. Bull. S. Calif. Acad. Sci. 19: 10. Ja 1920.

Davis, R. N. The winter aspect of trees. Am. For. **26**: 87–91. F 1920.

De Turk, E. Potassium-bearing minerals as a source of potassium for plant growth. Soil Science **8**: 269–301. 2 O 1919.

Detwiler, S. B. Results of white pine blister rust control in 1919. Phytopathology **10**: 177–180. 1920.

Dickie, F. Discovery of sugar on Douglas fir. Am. For. **26**: 84–86. F 1920.

Eaton, W. P. American wildflowers. Mentor **2**: 1–11. 15 Jl 1914.

Edson, H. A., & Shapovalov, M. Temperature relations of certain potato-rot and wilt-producing fungi. Jour. Agr. Research **18**: 511–524. *f. 1–9.* 16 F 1920.

Ehrhorn, E. M. New pests on the mainland. Hawaiian Forest. & Agr. **17**: 35–36. F 1920.
Flag smut and Fake-all diseases.

Elliott, J. A. Arkansas peach diseases. Bull. Univ. Arkansas Agr. Exper. Sta. 149: 1–9. *pl. 1–5*. Jl 1918.

Elmer, A. D. E. New woody plants from Mount Maquiling. Leafl. Philipp. Bot. **8**: 3069–3105. 22 O 1919.
39 new species.

Elmer, A. D. E. Zingiberaceae of the Sorsogon Peninsula. Leafl. Philipp. Bot. **8**: 2963–2995. 31 My 1919.
13 new species.

Erz, A. A. The true nature of plant diseases. Am. Bot. **26**: 20–23. 20 F 1920.

Fernald, M. L. Some variations of *Cardamine pratensis* in America. Rhodora **22**: 11–14. 28 F 1920.

Fernald, M. L. *Polygala paucifolia* Willd., forma *vestita*, n. f. Rhodora **22**: 32. 11 Mr 1920.

Fernald, M. L. The northern variety of *Ranunculus hispidus*. Rhodora **22**: 30–31. 11 Mr 1920.
Ranunculus hispidus falsus var. nov.

Fischer, H. Apogamie bei Farnbastarden. Ber. Deutsch. Bot. Gesellsch. **37**: 286–292. *f. 1*. 15 Jl 1919.

Fracker, S. B. Varietal susceptibility to false blossom in cranberries. Phytopathology **10**: 173–175. 1020.

Griggs, R. F. The effect of the eruption of Katmai on land vegetation. Bull. Am. Geog. Soc. **47**: 193–203. *f. 1–10*. Mr 1915.

Hackney, D. C. Some Texas wildflowers. Am. Bot. **26**: 19–20. 1920.

Harms, H. Uber die Blüteverhältnisse der bei uns im Freien angebauten Arten von *Forestiera* Poir. Mitteil. Deutsch. Dendr. Ges. **28**: 69–78. 1919.

Hartley, C., & Hahn, G. G. Notes on some diseases of aspen. Phytopathology **10**: 141–147. *f. 3*. 1920.

Herre, A. C. Notes on Mexican lichens. Bryologist **23**: 3–4. 24 Mr 1920.

Hitchcock, A. S. Botany in British Guiana. The Daily Argosy for Jan. 31, 1920. Georgetown, B. G.

Hitchcock, A. S. The genera of grasses of the United States with special reference to the economic species. Bull. U. S. Dept. Agr. 772: 1–288. *pl. 1–20* + *f. 1–174.* 20 Mr 1920.
1 new species and 14 new combinations.

House, H. D. The vegetation of the eastern end of Oneida Lake. Bull. N. Y. State Museum 197: 61–110. *f. 5–12.* 1916.
List of ferns, conifers and flowering plants.

Hutchinson, J. *Caulophyllum thalictroides.* A "gymnospermous" dicotyledon. Gard. Chron. **67**: 63. *f. 26.* 7 F 1920.

Jones, D. F. The effects of inbreeding and crossbreeding upon development. Bull. Conn. Agr. Exper. Sta. 207: 5–100. *pl. 1–12* + *f. 1–3.* S 1918.

Judd, C. S. The Koa tree. Hawaiian Forest. & Agr. **17**: 30–35. F 1920.

Kelley, W. P., & Thomas, E. E. The effects of alkali on citrus trees. Bull. Agr. Exper. Sta. Calif. 318: 305–337. Ja 1920.

Koehler, A. The identification of mahogany. Jour. For. **28**: 155–156. F 1920.

Kopeloff, N., & Byall, S. Invertase activity of mold spores as affected by concentration and amount of inoculum. Jour. Agr. Research **18**: 537–542. 16 F 1920.

Krebs, C. A rare Ohio plant immigrant. Am. Bot. **26**: 1–2. F 1920.
Berberis Aquifolium.

Latham, R. Musci hosts of *Cyphella muscigena* Fr. Bryologist **23**: 7. 24 Mr 1920.

Legrand, J. F. Plantas utiles de Puerto Rico. Rev. Agr. de Puerto Rico **4**: 5–10. 28 F 1920.

Liese, I. Uber den Heliotropismus der Assimilationszellen einiger Marchantiaceen. Ber. Deutschs. Bot. Gesellsch. **37**: 293–298. *f. 1–4.* 25 Jl 1919.

Lingelsheim, A. Notizen über *Fraxinus.* Mitteil. Deutsch. Dendr. Gesellsch. **28**: 78–82. 1919.

Lloyd, C. G. Mycological notes 61: 877–903. *pl. 124–139.* O 1919.

Lloyd, J. U. Colorophyl complexities. Am. Jour. Phar. **92**: 110–114. F 1920.

Loeb, J. The nature of the directive influence of gravity on the arrangement of organs in regeneration. Jour. Gen. Physiol. **2**: 373–386. *f. 1–9.* 16 Ja 1920.

Loesener, T. Uber die Aquifoliaceen, besonders über *Ilex*. Mitteil. Deutsch. Dendr. Gesellsch. **28**: 1–66. *pl. 1–4.* 1919.

Lorenz, A. Some Hepaticae from Matinicus Island, Maine. Bryologist **23**: 1–3. 24 Mr 1920.

Lowe, R. L. *Rhacomitrium sudeticum*, a moss new to Worcester County, Massachusetts. Bryologist **23**: 4–5. 24 Mr 1920.

MacDougal, D. T. Hydration and growth.
Carnegie Inst. Washington, Publ. 297. 1–176. *f.* 1–52. 1920.

Matsumoto, T. Culture experiments with *Melampsora* in Japan. Ann. Mo. Bot. Gard. **6**: 309–316. *f. 1–3.* 1920.

Mayes, J. Sixty troublesome weeds. Bull. Missouri Board Agr. **17**: 2–47. D 1919.

McCulloch, L. Basal glumerot of wheat. Jour. Agr. Research **18**: 543–551. *pl. 62–63.* 16 F 1920.

Melchers, L. E., & Parker, J. H. Three winter wheat varieties resistant to leaf rust in Kansas. Phytopathology **10**: 164–171. *f. 1–3.* 1920.

Merrill, E. D. Additional notes on the Kwangtung flora. Philipp. Jour. Sci. **15**: 225–261. 1919.
14 new species and many new combinations.

Meyer, R. *Echinopsis gigantea* R. Mey., sp. nov. Monats. Kakteenk. **29**: 58–60. May 1919.

Miller, R. B. The wood of *Machaerium Whitfordii*. Bull. Torrey Club **47**: 73–79. *f. 1–8.* 10 Mr 1920.

Moore, G. T., & Karrer, J. L. A subterranean algal flora. Ann. Missouri Bot. Gard. **6**: 281–307. 1920.

Murrill, W. A. The artist's bracket fungus. Sci. Am. **122**: 365. 1920.

Neal, D. C. Phony peaches; a disease occurring in middle Georgia. Phytopathology **10**: 106–109. *pl. 9.* + *f. 1.* 1920.

Nelson, J. C. A noteworthy grass. Am. Bot. **26**: 10–12. 20 F 1920.
Coleanthus subtilis.

Nutting, C. C. Barbados-Antigua Expedition. Univ. Iowa Stud. Nat. Hist. **8**: 219–223. My 1919.

Orton, W. A. Streak disease of potato. Phytopathology **10**: 97–100. *pl.* 8 + *f.* 1. 1920.

Peltier, G. S. Snapdragon rust. Bull. Univ. Illinois Agr. Exper. Sta. 221: 535–548. *f.* 1–5. Au 1919.

Peterson, W. H., & Fred, G. B. Fermentation of fructose by *Lactobacillus pentoaceticus*, n. sp. Jour. Biol. Chem. **41**: 431–450. *f.* 1–2. 20 Ja 1920.

Purpus, J. A. *Pachyphytum oviferum* J. A. Purpus, nov. spec. Monats. Kakteenk. **29**: 100–103. S 1919.

Ramaley, F. Subalpine lake-shore vegetation in north-central Colorado. Am. Jour. Bot. **7**: 57–73. *f.* 1–6. 10 Mr 1920.

Rand, F. V., & Cash, L. C. Some insect relations of *Bacillus tracheiphilus* Erw. Sm. Phytopathology **10**: 133–140. *f.* 1. 1920.

Robbins, W. W., & Boyack, B. The identification and control of Colorado weeds. Bull. Colorado Agr. Exper. Sta. 251: 5–123. Jl 1919.

Roberts, W. American plants in England. Gard. Chron. **67**: 52. 7 F 1920.

Rose, D. H., Kraybill, H. R., & Rose, R. C. Effect of salts upon oxidase activity of apple bark. Bot. Gaz. **69**: 218–236. *f.* 1–5. 16 Mr 1920.

Rosen, H. R. A bacterial root-rot of field corn. Bull. Univ. Arkansas Agr. Exper. Sta. 162: 1–6. *pl.* 1–4. Au 1919.

Rosenbaum, J. Infection experiments on tomatoes with *Phytophthora terrestria* Sherb., and a hot water treatment of the fruit. Phytopathology **10**: 101–105. 1920.

Rosenbaum, J., & Sando, C. E. Correlation between size of the fruit and the resistance of the tomato skin to puncture and its relation to infection with *Macrosporium tomato* Cooke. Am. Jour. Bot. **7**: 78–82. 10 Mr 1920.

Rumbold, C. Effect on chestnuts of substances injected into their trunks. Am. Jour. Bot. **7**: 45–56. *pl.* 3–4. 10 Mr 1920.

St. John, H. The genus *Elodea* in New England. Rhodora **22**: 17–29. 11 Mr 1920.
2 new combinations.

Savelli, R. Contribuzione allo studio della pistillodia ovulare. Ann. di Bot. **15**: 1–27. *pl.* 1. 31 Ja 1920.

Sayre, J. D. The relation of hairy leaf coverings to the resistance of leaves to transpiration. Ohio Jour. Sci. **20**: 55–86. *f.* 1–7. Ja 1920.

Schwerin, F., Graf von. Zur Ehrenrettung des *Acer Negundo.* Mitteil. Deutsch. Dendr. Gesellsch. **28**: 146–150. 1919.

Schwerin, F., Graf von. Die Bedeutung der Bauwelt Chiles für Deutschland. Mitteil. Deutsch. Dendr. Gesellsch **28**: 121–131. *pl. 7–10.* 1919.

Schwerin, F., Graf von. Dendrologische Notizen. Mitteil. Deutsch. Dendr. Gesellsch. **28**: 168–189. 1919.

Schwerin, F., Graf von. Uber Küstenklima. Mitteil. Deutsch. Dendr. Gesellsch. **28**: 116–120. 1919.

Schwerin, F., Graf von. Angeblicher Atavismus bei *Liriodendron.* Mitteil. Deutsch. Dendr. Gesellsch. **28**: 135–143. 1919.

Schwerin, F., Graf von. Versuch einer Erklärung merkwürdiger Pflanzenbewegungen. Mitteil. Deutsch. Dendr. Gesellsch. **28**: 160–167. *pl. 11.* 1919.

Sharp, L. W. Spermatogenesis in *Blasia.* Bot. Gaz. **69**: 258–268. *pl. 15.* 1920.

Soth, B. H. Wildflower distribution in the West. Am. Bot. **26**: 14–16. 20 F 1920.

Standley, P. C. Sphagnum in Glacier National Park, Montana. Bryologist **23**: 5–6. 24 Mr 1920.

Stevens, F. L. Dothidiaceous and other Porto Rican Fungi. Bot. Gaz. **69**: 248–257. *pl. 13, 14.* + *f. 1–3.* 16 Mr 1920.
1 new genus, 15 new species.

Stevenson, J. A. The mottling or yellow stripe disease of sugar cane. Jour. Dept. Agr. Porto Rico **3**: 3–76. Jl 1919.

Thomas, R. C. A new lettuce disease. Ohio Agr. Exper. Sta. Monthly Bull. **5**: 24–25. Ja 1920.

Tisdale, W. B. Iris leaf spot caused by *Didymellina Iridis.* Phytopathology **10**: 148–163. *f. 1–6.* 1920.

Vasey, H. E. Millet smuts and their control. Colorado Agr. Exper. Sta. Bull. 242: 3–22. *f. 1–11.* F 1918.

Von Oven, F. W. Perpetuating our native flora. Am. Bot. **26**: 24–27. 20 F 1920.

Walker, J. C., & Tisdale, W. B. Observations on seed transmission of the cabbage black rot organism. Phytopathology **10**: 175–177. 1920.

Wann, F. B. The fixation of free nitrogen by green plants. Science II. **61**: 247–248. 5 Mr 1920.

Weatherby, C. A. *Habenaria psycodes* var. *ecalcarata* in Vermont. Rhodora **22**: 31–32. 11 Mr 1920.

Weimer, J. L. Some observations on the spore discharge of *Pleurage curvicolla* (Wint.) Kuntze. Am. Jour. Bot. **7**: 75–77. 10 Mr 1920.

Weimer, J. L. The distribution of buckeye rot of tomatoes. Phytopathology **10**: 172. 1920.

Weingart, W. Die Blüte des *Cereus aurivillus* K. Sch. Monats. Kakteenk. **29**: 6–10. Ja 1919.

Weingart, W. *Cereus repandus* Haw. und Verwandte. Monats. Kakteenk. **29**: 85–93. Au 1919.

Weingart, W. *Cereus ruber* Weing. Monats. Kakteenk **29**: 57–58. My 1919.

Weingart, W. Frucht des *Cereus serratus* Weing. Monats. Kakteenk. **29**: 93–94. Au 1919.

Weingart, W. Vom Reif des *Cereus trigonus* Haw. var. *guatemalensis* Eichl. Monats. Kakteenk. **29**: 80–84. Jl 1919.

Wicks, W. H. Effect of cross-pollination on size, color, shape and quality of the apple. Bull. Univ. Arkansas Agr. Exper. Sta. 143: *pl. 1–9.* Mr 1918.

Wiegand, K. M. A new species of *Spergularia*. Rhodora **22**: 15–16. 28 F 1920.

Wiegand, K. M. Variations in *Lactuca canadensis*. Rhodora **22**: 9–11. 28 F 1920.

Wolf, F. A. A bacterial leaf spot of velvet bean. Phytopathology **10**: 73–80. *f. 1–2.* 1920.
Aplanobacter Stizolobii n. sp.

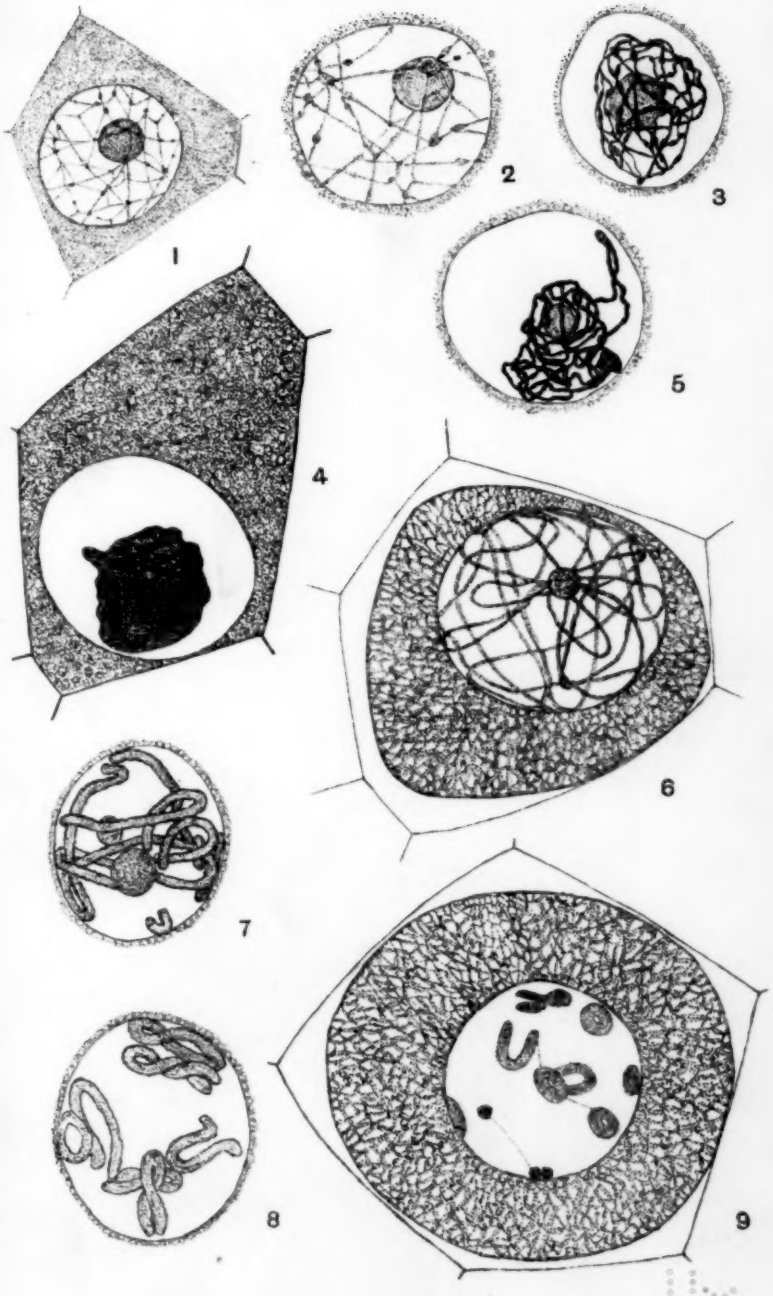
Wolf, F. A. Bacterial blight of soybean. Phytopathology **10**: 119–132. *f. 1–5.* 1920.

Woolsey, T. S. Natural regeneration of French forests. Am. For. **26**: 77–81. F 1920.

Wright, G. Pit-closing membrane in Ophioglossaceae. Bot. Gaz. **69**: 237–247. *pl. 11, 12.* + *f. 14–19.* 16 Mr 1920.

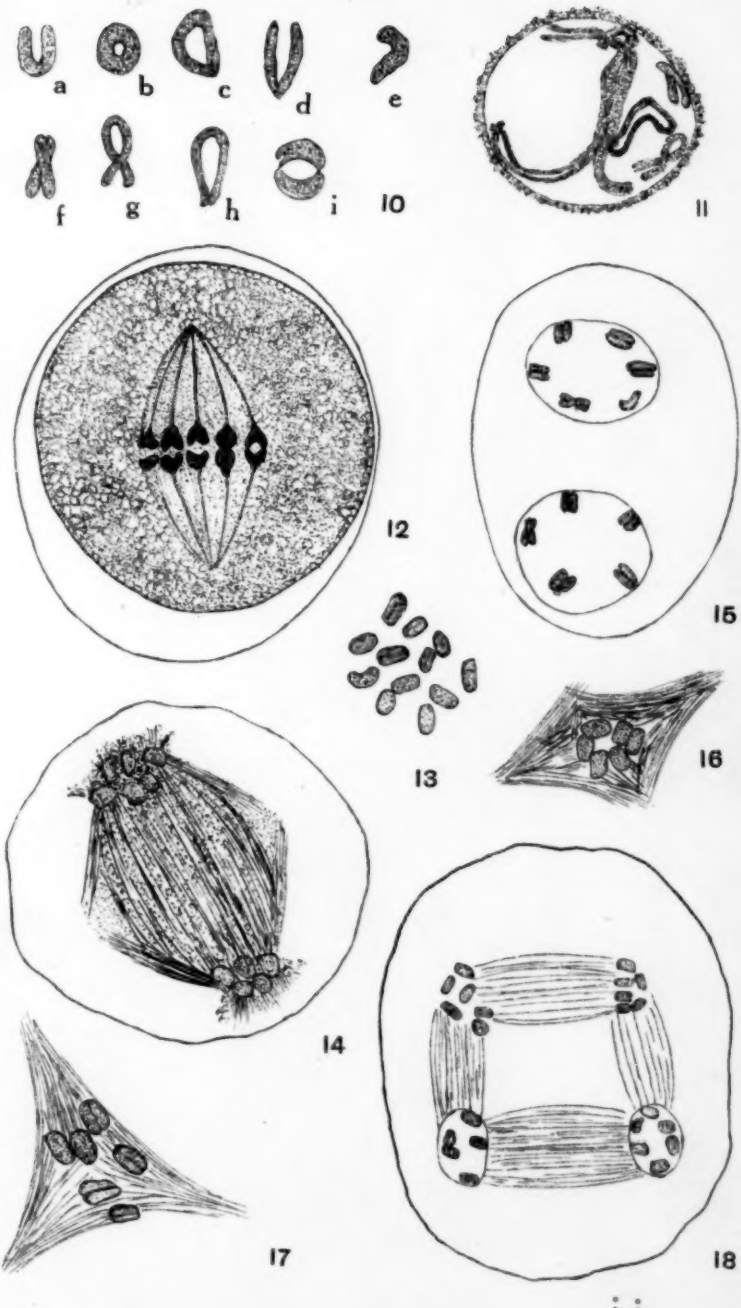
York, H. H. Late seasonal production of aecia of *Cronartium ribicola*. Phytopathology **10**: 111. 1920.

Zinn, J. On variation in Tartary buckwheat, *Fagopyrum tataricum* (L.) Gaertn. Genetics **4**: 534–586. *f. 11.* N 1919.



O NEAL: MICROSPOROGENENSIS IN DATURA

HELIOTYPE CO. BOSTON



O'NEAL: MICROSPOROGENENSIS IN DATURA